JACC Vol. 8, No. 2
August 1986:349–56

Beta-Adrenergic Receptor Properties of Canine Myocardium: Effects of Chronic Myocardial Infarction

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To determine the effects of chronic myocardial infarction on beta-adrenergic properties of canine myocardium, the hearts of nine mongrel dogs were studied 3 weeks after acute myocardial infarction. Infarction was produced by ligating the left anterior descending coronary artery in five dogs and the circumflex artery in four dogs. The heart was divided into normal and infarct zones (either anterior or posterior, depending on the vessel ligated) and marginal zones (septal and lateral), each zone being subdivided into epicardial and endocardial portions. Myocardial blood flow (microsphere technique) was markedly reduced in the infarct zone.

In eight endocardial infarct samples after left anterior descending ligation, the maximal number (± SD) of binding sites assessed by ^{125}I-iodocyanopindolol was 3.9 ± 1.9 pmol/mg deoxyribonucleic acid (DNA) and was reduced from normal endocardial values (9.7 ± 9.4 pmol/mg DNA, $p < 0.05$). The dissociation constant (K_d), which is a measure of the affinity of the iodinated antagonist for the receptor, did not differ (304 ± 222 versus 338 ± 219 p*M*, p = NS). In the epicardium, the maximal number of beta-adrenergic receptors was also reduced ($p < 0.05$), without a change in K_d. In the lateral and septal zones neither the maximal number of binding sites nor K_d values differed from those of normal endocardium. In nine endocardial infarct zones, (−)-isoproterenol-stimulated adenylate cyclase activity was reduced compared with control (34,870 ± 29,430 versus 88,660 ± 63,640 pmol/mg DNA/30 minutes, $p < 0.01$), but the ratio of (−)-isoproterenol-stimulated to maximal (sodium fluoride-stimulated) adenylate cyclase activity was unchanged between normal and infarct zones. No other changes from normal were found in any of the other zones. Three weeks after circumflex artery ligation, there were no significant differences in beta-adrenergic receptor density or affinity or in (−)-isoproterenol-stimulated or maximal adenylate cyclase activity when normal and infarct zones were compared.

It is concluded that after chronic canine myocardial infarction there is no evidence of increased beta-adrenergic receptor density or augmented (−)-isoproterenol-stimulated adenylate cyclase activity. Therefore, any alterations in beta-adrenergic receptor properties after myocardial infarction must occur rapidly or be mediated by a pathway other than direct alteration of the beta-adrenergic receptor or coupling between the receptor and adenylate cyclase.

(J Am Coll Cardiol 1986;8:349–56)

Primarily on the basis of two large clinical trials (1,2), long-term beta-adrenergic blockade has been recommended to prevent recurrent infarction and sudden death after acute myocardial infarction. The mechanism of action of beta-adrenergic blockade under these circumstances is unknown. Proposed mechanisms include suppression of arrhythmias or ischemia produced by augmented catecholamine levels, reduction in myocardial oxygen requirements or favorable effects on myocardial blood flow distribution or metabolism (3–5). Virtually all previous experimental studies of beta-adrenergic receptor properties in ischemia have been acute (1 to 3 hours) (6–8) or subacute (3 days) (9). Therefore we decided to measure myocardial blood flow, $beta_1$-adrenergic receptor density and affinity and adenylate cyclase activity late (3 weeks) after acute canine myocardial infarction.

The goal of this study was to determine whether there are long-term alterations in beta-adrenergic receptor density or adenylate cyclase activity, or both, after myocardial infarction. If either an increase in signal transduction manifested as increased enzyme activity or alterations in receptor

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Manuscript received December 10, 1985; revised manuscript received February 11, 1986, accepted March 3, 1986.

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0735-1097/86/$3.50

density could be demonstrated, an additional rationale would be provided for the use of long-term beta-adrenergic blockade after myocardial infarction.

Methods

Experimental preparation. Seventeen mongrel dogs weighing 20 to 25 kg were studied. Eight of them died suddenly in the immediate postoperative period. Of the nine that survived, five underwent ligation of the left anterior descending coronary artery to produce an anterior wall myocardial infarction and four underwent ligation of the circumflex coronary artery to produce a posterior wall infarction. These nine dogs all survived for 3 weeks, after which they were killed and the heart removed for study.

At the start of the study, each dog underwent a left thoracotomy under 1% halothane general anesthesia, and the coronary arteries and left atrium were exposed through a small pericardial incision. After control microsphere measurement of myocardial blood flow, either the left anterior descending (n = 5) or the circumflex (n = 4) coronary artery was ligated to produce a large acute myocardial infarction, and the thoracotomy incision was closed. At the time of ligation, lidocaine (1 to 2 mg, intravenously) was given and each day for 1 week postoperatively, procainamide (25 mg/kg body weight intramuscularly) was administered to reduce the incidence of ventricular arrhythmias. Three weeks after the initial ligation, each of the nine surviving dogs again underwent a left thoracotomy under halothane anesthesia. After repeat measurement of blood flow, the heart was immediately removed and placed in iced 50 m*M* Tris buffer, pH 7.4, before sectioning.

Preparation of myocardial zones. In the first four dogs, the left ventricular free wall and septum were immediately cut, parallel to the atrioventricular groove, into a large central portion and smaller apical and basal rings. The central portion, which encompassed the infarct area, was subdivided into three rings of equal thickness (Fig. 1). Each circular section was then divided into four quadrants: infarct, adjacent medial and lateral zones and a normal zone opposite to the area of the infarct. Care was taken during sectioning to avoid contamination of the infarct zone with adjacent normal-appearing tissue. Each zone was then subdivided into endocardial and epicardial regions, weighed and minced with scissors. Apical and basal cross sections were utilized for measurement of adenylate cyclase activity and for beta-adrenergic receptor binding studies, and the intervening center section was used for coronary blood flow measurement (Fig. 1).

In the remaining five dogs, including all four dogs with circumflex coronary artery ligation, a large cross section of the left ventricle and septum was removed. This cross section encompassed the epicardial extent of the infarct and was equivalent to the basal, central and apical sections of the hearts in the first four dogs. This large section was divided into the previously described four quadrants and then into endocardial and epicardial regions. After these fractions were minced with scissors, aliquots were taken from each tissue sample, weighed and randomly assigned for measurement of myocardial blood flow, beta-adrenergic receptor binding studies and assay of adenylate cyclase activity.

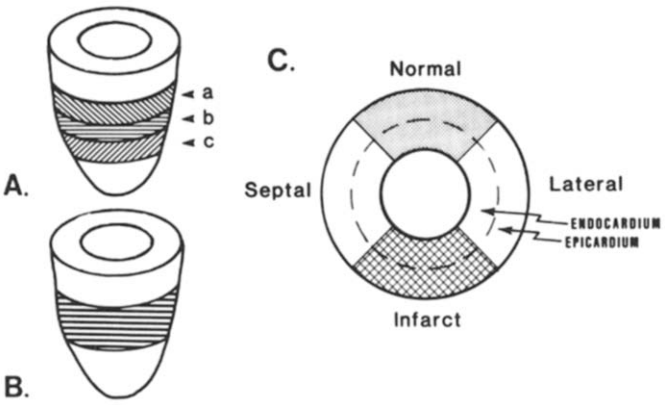


Figure 1. Myocardial samples and zones. **A**, Left ventricle. **a** and **c**, Cross sections of myocardium removed for beta-adrenergic receptor studies and adenylate cyclase assay (four dogs). **b**, Cross section of myocardium removed for myocardial blood flow determination (four dogs). **B**, Large cross section of myocardium removed for determination of myocardial blood flow, beta-adrenergic receptor studies and adenylate cyclase assays (five dogs). **C**, Cross section of myocardium depicting the zones of myocardium that were analyzed. See text for details.

Preparation of myocardial samples. For the latter analyses, the minced tissue samples from endocardial and epicardial areas and from each of the zones described were homogenized in 0.25 *M* sucrose, 1 μM ethylenediaminetetraacetic acid and 5 m*M* Tris-hydrochloride (pH 7.4) and centrifuged at 270*g* for 15 minutes. The supernatant was then pelleted at 41,300*g* for 15 minutes and washed two additional times in the same buffer and frozen in liquid nitrogen. The samples were stored at −70°C until analysis several weeks later. Preliminary studies showed that there is no loss of either receptor binding or adenylate cyclase activity under these circumstances, compared with fresh tissue.

Myocardial blood flow measurement. For each measurement of myocardial blood flow, radioactive microspheres (average mean diameter [±SD] 15 ± 2 μm) were labeled with gadolinium-153, cobalt-57, chromium-51, tin-113, strontium-85, niobium-95, indium-114 or zinc-65. Using the reference sample technique (10), 1 to 2 million microspheres were injected into the left atrium after thorough agitation. Reference sample collection from a distal aortic catheter was begun 5 seconds before injection and continued for 2 minutes, using a Holter pump at a constant withdrawal rate of 15 ml/min. Injection of microspheres had

no effect on heart rate or mean aortic pressure in any dog. A least squares radionuclide separation technique was used for determination of regional myocardial blood flow (11). Under these circumstances, there is minimal short- and long-term loss of microspheres from canine left ventricular myocardium (12).

Radioligand binding studies and adenylate cyclase activity. For beta$_1$-adrenergic receptor analyses after left anterior descending artery ligation, [^{125}I]iodocyanopindolol was used (13), and membrane preparations obtained after circumflex artery ligation were incubated with [^{3}H]dihydroalprenolol, as previously described (14). Nonspecific binding was defined as that component of total binding not inhibited by 1 μM (−)-propranolol, and it did not exceed 20% of total binding for both radioligands.

All radioligand binding studies were carried out in triplicate. Analysis of saturation binding isotherms was performed according to the method of Scatchard (15). Protein was determined by the method of Lowry et al. (16), using bovine serum albumin as standard, and deoxyribonucleic acid (DNA) content was measured by the method of Hinegardner (17). Adenylate cyclase activity was determined by the method of Salomon et al. (18). Maximal adenylate cyclase activity was assessed by measuring cyclic adenosine monophosphate production in the presence of 10 mM sodium fluoride and 0.1 mM guanyl-5′-yl imidodiphosphate.

Statistical analysis. All data are expressed as mean ± SD. For comparisons, Student's t test for paired or unpaired data was utilized, as appropriate.

Results

Myocardial blood flow studies. Adequate estimates of myocardial blood flow were obtained in six dogs (two with left anterior descending and four with circumflex artery ligation). In the preligation studies there were no significant differences between endocardial and epicardial flows, either in the control area or in the areas destined to undergo myocardial infarction (infarct baseline). Three weeks after ligation the infarct areas showed a marked decline in myocardial blood flow compared with flow in the opposite control wall (Fig. 2). There were no differences between infarct zones produced by left anterior descending or circumflex artery ligation.

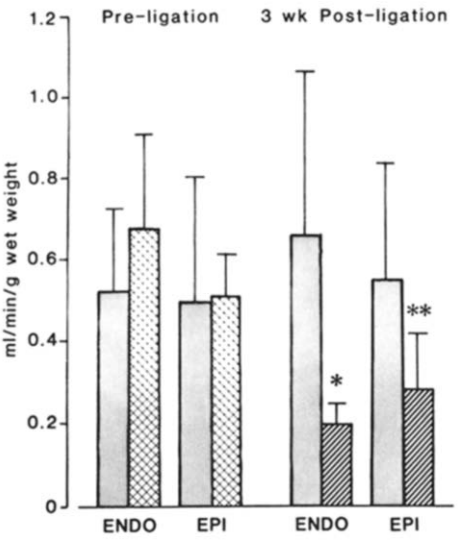


Figure 2. Myocardial blood flow in six dogs determined by the microsphere technique. The persistence of blood flow in the infarct area is presumably due to collateral flow. **Shaded bars** = control baseline; **crosshatched** and **hatched bars** = infarct area; **vertical T bars** = 1 SD. *$p < 0.005$ versus control baseline; **$p < 0.02$ versus control baseline. ENDO = endocardial; EPI = epicardial; n = number of dogs; SD = standard deviation.

Radioligand binding studies (Table 1). Beta-adrenergic receptor density and affinity were measured in eight endocardial samples and in six epicardial samples from five dogs that had undergone left anterior descending artery ligation. The equilibrium dissociation constant (K_d) of the receptors for the radioligand is the amount of radioligand required to bind to half the receptors. It is measured in units

Table 1. Radioligand Binding Studies

		Bmax (fmol/mg DNA)		K_d	
	n	Normal	Infarct	Normal	Infarct
Left Anterior Descending Artery Ligation					
Endocardium	8	9.7 ± 9.4	3.9 ± 1.9*	338 ± 219 pM	304 ± 222 pM
Epicardium	6	13.8 ± 13.7	7.2 ± 5.9*	326 ± 163 pM	386 ± 269 pM
Circumflex Artery Ligation					
Endocardium	4	7.5 ± 3.3	15.3 ± 12.7	5.2 ± 1.6 nM	7.3 ± 3.9 nM
Epicardium	4	14.8 ± 6.7	11.0 ± 3.1	7.5 ± 4.7 nM	6.1 ± 2.0 nM

*$p < 0.05$ versus normal myocardium. All data are mean values ± 1 SD. Bmax = maximal number of binding sites; DNA = deoxyribonucleic acid; fmol = femtomoles; K_d = equilibrium binding dissociation constant; n = number of samples.

of concentration and is inversely related to the affinity or "strength" with which the receptor binds to the ligand; that is, high affinity = low K_d and vice versa (19). There was no difference in K_d values between endocardium and epicardium or between normal and infarct zones. However, after chronic myocardial infarction there was a decline in $beta_1$-adrenergic receptor density in the infarct zone when data were expressed per milligram deoxyribonucleic acid (DNA) content. When protein determinations were utilized to normalize the data, there was also a decrease in $beta_1$-adrenergic receptor density in the infarct zone, but this value did not achieve statistical significance. Septal and lateral areas showed no difference either in K_d or in the maximal number of binding sites compared with the normal areas, regardless of the method of normalization (n = 6, data not shown). Similarly, for circumflex artery ligation K_d values showed no differences between endocardium and epicardium or between normal and infarct areas. Also there were no significant differences in the maximal number of binding sites, whether normalized per milligram DNA or per milligram protein.

Adenylate cyclase activity. After left anterior descending artery ligation, the reduction in beta-adrenergic receptor density was accompanied by a decline in maximally stimulated adenylate cyclase activity as shown by the 45% reduction in enzyme activity after incubation with sodium fluoride. Similarly, activity stimulated by the nonhydrolyzable analog of guanosine triphosphate, guanyl-5′-yl imidodiphosphate, was reduced by 37% in the endocardial layer when infarcted and normal tissue were compared (Fig. 3). In the epicardium maximal enzyme activity was also reduced by 29%; guanyl-5′-yl imidodiphosphate-stimulated activity was also reduced, but this reduction was not statistically significant (Fig. 3). These results and the results described later were similar whether the data were normalized per milligram DNA or per milligram protein.

Figure 4 depicts the adenylate cyclase activity stimulated by (−)-isoproterenol after left anterior descending artery ligation. Because canine myocardial membranes respond poorly to (−)-isoproterenol stimulation alone at 1 μM, we measured what we have called net (−)-isoproterenol stimulation by subtracting the guanyl-5′-yl imidodiphosphate-stimulated activity from a combination of the latter plus 1 μM (−)-isoproterenol-stimulated activity. This is similar to the approach used by Marsh et al. (20) in chick ventricular myocardial tissue. As Figure 4 demonstrates, along with the previously noted reduction in $beta_1$-adrenergic receptor density and sodium fluoride-stimulated adenylate cyclase activity, there was a significant 61% reduction in net (−)-isoproterenol-stimulated adenylate cyclase activity in the infarct area in the endocardial zone. There was, however, no significant difference in the epicardium. When the ratios of sodium fluoride-stimulated activity to net (−)-isoproterenol-stimulated activity were compared, however, there were no significant differences in either the endocardial or the epicardial zone. Thus the reduction in the net (−)-isoproterenol-stimulated activity seems to be a consequence of an overall decline in the endocardial activity of the enzyme in the infarct zone.

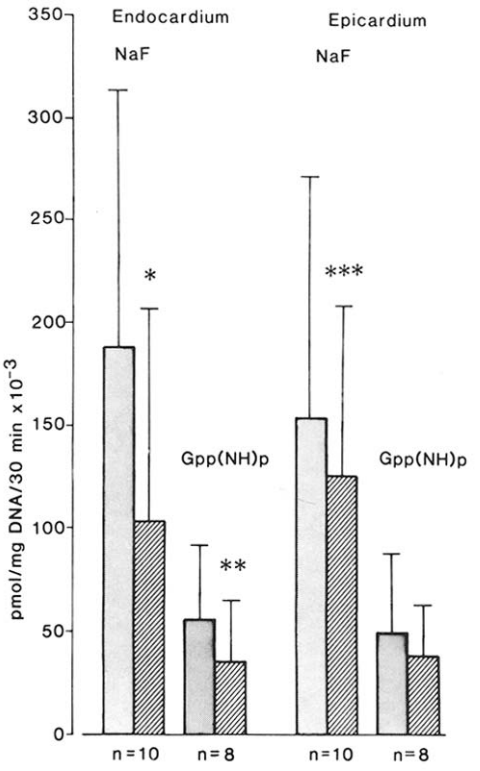
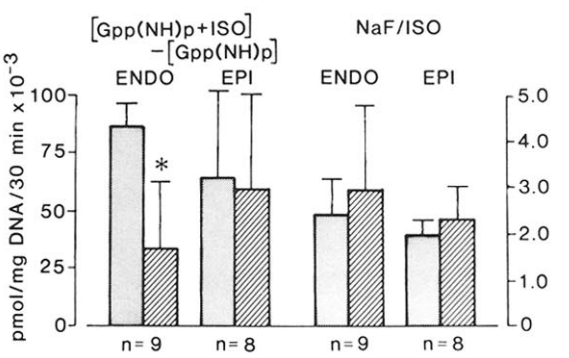


Figure 3. Adenylate cyclase activity stimulated by sodium fluoride and guanyl-5′-yl imidodiphosphate [Gpp(NH)p] after left anterior descending artery ligation. DNA = deoxyribonucleic acid; n = number of samples analyzed from five dogs; NaF = sodium fluoride; SD = standard deviation. **Shaded bars** = normal area; **hatched bars** = infarct area; vertical T bars = 1 SD. *$p < 0.0125$; **$p < 0.025$; *** $p < 0.05$.

Figure 4. Left, Net (−)-isoproterenol (ISO)-stimulated adenylate cyclase activity calculated as [Gpp(NH)p + ISO] − [Gpp(NH)p] after left anterior descending artery ligation. **Right,** Ratio of maximal (sodium fluoride-stimulated) to net (−)-isoproterenol (ISO)-stimulated adenylate cyclase activity. **Shaded bars** = normal area; **hatched bars** = infarct area; **vertical T bars** = 1 SD. *$p < 0.01$. Abbreviations as in Figures 2 and 3.



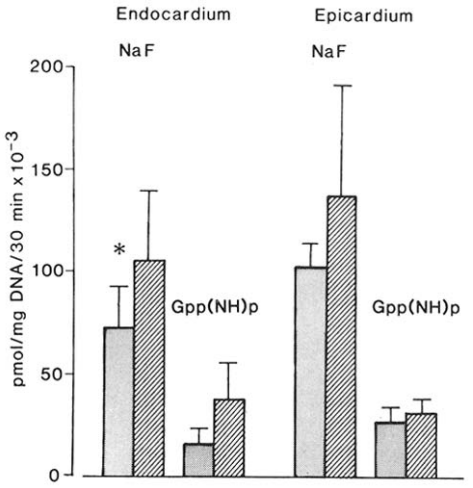
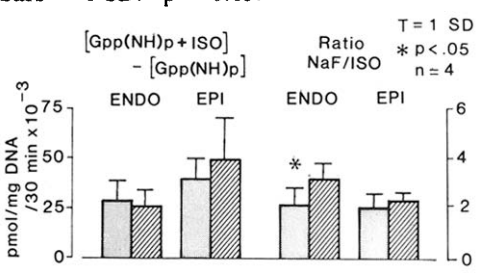


Figure 5. Adenylate cyclase activity stimulated by sodium fluoride (NaF) and guanyl-5′-yl imidodiphosphate [Gpp(NH)p] after circumflex artery ligation in four dogs. Other abbreviations as in Figure 3. **Shaded bars** = normal area; **hatched bars** = infarct area; **vertical T bars** = 1 SD. $p < 0.05$.

After circumflex coronary artery ligation, there was a 44% increase in sodium fluoride-stimulated adenylate cyclase activity in the endocardial zone and no difference in the epicardium (Fig. 5). The changes in guanyl-5′-yl imidodiphosphate-stimulated adenylate cyclase activity in both the endocardium and the epicardium were not different. Net (−)-isoproterenol-stimulated activity in the infarct zone, whether measured in the endocardium or epicardium, was not different from that in control areas (Fig. 6). Indeed, the ratio of sodium fluoride-stimulated to net (−)-isoproterenol-stimulated activity increased somewhat in the infarct zone compared with the normal zone, indicating a relative decline in catecholamine responsiveness, whereas in the epicardium there was no significant difference in this ratio.

Adenylate cyclase activity stimulated by sodium fluoride, guanyl-5′-yl imidodiphosphate and net (−)-isoproterenol-stimulated activity was not different from control areas in the epicardial or endocardial septal or lateral zones (n = 8, data not shown).

Figure 6. Left, Net (−)-isoproterenol (ISO)-stimulated adenylate cyclase activity calculated as [Gpp(NH)p + ISO] − [Gpp(NH)p] after circumflex artery ligation. **Right,** Ratio of maximal (sodium fluoride-stimulated) to net (−)-isoproterenol (ISO)-stimulated adenylate cyclase activity. Abbreviations as in Figures 2 and 3. **Shaded bars** = normal area; **hatched bars** = infarct area; **vertical T bars** = 1 SD. *$p < 0.05$.



Discussion

Clinical experience. Two large clinical trials (1,2) in human subjects have suggested that beta-adrenergic blockade begun 5 to 7 days after acute myocardial infarction may be useful in preventing recurrent infarction and sudden death. The results of other clinical trials (21–24) involving smaller numbers of patients have also supported the use of beta-blockade after myocardial infarction. However, there has been some dispute regarding the routine use of beta blockers after myocardial infarction, particularly in patients who have had an uncomplicated course (25). Indeed the results of the Beta-Blocker Heart Attack Trial demonstrated that the major benefit occurred in patients who had a complicated course and that long-term beta-blockade was of relatively little value in individuals who had an uncomplicated myocardial infarction or a non-Q wave infarct (2,26).

Mechanism of beta-blockade. The mechanism of action of beta-blockers in preventing a recurrent infarction or sudden death is unknown. After 1 hour of acute myocardial ischemia in a canine model, there was an increase in beta-adrenergic receptor density without a change in affinity, and after 15 minutes of reperfusion there was an increase in adenylate cyclase activity stimulated by isoproterenol (6,7). The increase in beta-adrenergic receptor density appears to result largely from an increase in beta-receptors on cardiac myocytes rather than on blood vessels (8). Other studies in the rat (9) have suggested a reduction in beta-receptor density and adenylate cyclase response in normal myocardium 3 days after myocardial infarction, and in studies in the cat (27,28) a reduction in catecholamine stores and in contractile activity occurs in noninfarcted areas; these reductions return to normal levels 6 weeks after infarction. However, to our knowledge, no previous investigations have addressed the issue of whether there are alterations in beta-adrenergic receptor properties or signal transduction after chronic myocardial infarction. Also, in previous short-term studies, endocardial versus epicardial changes were not considered, nor was myocardial blood flow measured.

Decreased adenylate cyclase activity and beta-receptor density. We hypothesized that if beta-adrenergic blockade is useful after chronic myocardial infarction, one mechanism of action might be that it reduces augmented adenylate cyclase activity, possibly as a result of an increased number of beta-adrenergic receptors in the infarct area or in the peri-infarction zone. The results of our study indicate that neither in the infarct zone itself nor in the myocardium immediately adjacent to the infarct area is there an increase in beta-receptor density or isoproterenol-stimulated adenylate cyclase activity. Indeed, after left anterior descending artery ligation there appeared to be a reduction in beta-adrenergic

receptor density in the infarct zone, accompanied by a general reduction in adenylate cyclase activity in this area. Our data suggest that the reduction in the net (−)-isoproterenol-stimulated activity is a result of this overall decline in enzyme activity. Such a selective reduction of enzyme activity, however, does not imply a lack of response to catecholamines in areas of depressed myocardium adjacent to the infarct zone (29,30). Our observation that adenylate cyclase activity and beta-adrenergic receptor density were normal in the septal and lateral zones is consistent with both experimental and clinical observations regarding the normal perfusion (29) and potential for augmented contractility (30) of these zones.

Similar changes were not demonstrable after myocardial infarction produced by circumflex coronary artery ligation, where beta-receptor density and adenylate cyclase responses were largely unchanged compared with findings in the opposite normal anterior wall. Whether these differences can be accounted for, at least in part, by regional differences in beta-adrenergic receptor density in the canine myocardium is unknown (31). These differences are unlikely to be due to the different tissue sampling techniques used, because the reduction in myocardial blood flow, beta-adrenergic receptor density and adenylate cyclase activity were similar in the dogs with left anterior descending artery ligation regardless of the method used to obtain the tissue.

Study limitations. Our study has certain limitations. Arrhythmias triggering sudden death after chronic myocardial infarction could still occur in a small border zone surrounding the infarct area (21,32,33). The technique we used to section the myocardium was not designed to identify such small, abrupt transition areas, which could possibly be involved in the pathogenesis of fatal arrhythmias in human subjects. The decreased coronary blood flow we measured is reported per gram wet weight of tissue, so that the actual flow to peninsulas of myocardium could have been normal. However, the decreased beta-adrenergic receptor density and diminished adenylate cyclase activity, particularly in the infarct area produced by left anterior descending ligation, suggest an abnormality of adrenergic function that could be the result of diminished collateral blood flow to that area (34). Although dogs have more normally occurring collateral flow and a greater increase in collateral flow after coronary occlusion than have humans, in our experiments the flow to the infarct zone was markedly reduced (Fig. 2). Such a long-term alteration in perfusion has been described by Arani et al. (35), who observed reduced coronary blood flow under rest conditions in collateral-dependent myocardium of patients with complete occlusion of the left anterior descending coronary artery. In another animal model (36), chronic hypoxia produced a decrease in both beta-adrenergic receptor density and adenylate cyclase activity in response to isoproterenol.

In the Beta-Blocker Heart Attack Trial (2), the major benefit was in patients with mechanical or electrical complications as opposed to patients with uncomplicated infarction. At the time of study, no dog showed evidence of congestive cardiac failure as manifested by dyspnea, edema, lethargy or weight loss. No pleural infection or effusions were noted, and the lungs appeared normal on gross inspection. Although each dog studied exhibited substantial scar formation in the area of the infarct, it is possible that this experimental model did not produce enough myocardial damage to cause alterations resembling those that occur in severe infarction in humans. We recognize that sudden infarction produced in a dog by coronary artery ligation may not mimic the situation that occurs in humans, where chronic coronary artery obstruction usually precedes infarction. Nevertheless, 3 weeks after infarction, when we performed our studies, the hearts exhibited chronic scar formation and peninsulas of viable myocardium with some residual blood flow, which to a substantial extent resembles the situation observed in humans.

Another consideration is the method used to normalize the data. Histologic sections of the infarct zone showed areas of normal appearing myocardium surrounded by large amounts of scar tissue containing scattered fibroblasts which could have accounted for only small and probably equivalent amounts of the protein and DNA content of the tissue samples. The protein assay we employed (16) does not measure the predominant amino acids in collagen (proline and hydroxyproline). In addition, collagen contains no DNA, and normalization per milligram DNA yielded virtually identical results to the data normalized per milligram protein, suggesting that for both methods, only viable myocardium in the infarct zone was being measured.

Possible mechanisms not examined by the present study. Our results do not exclude the possibility that the potentially beneficial effects of beta-adrenergic blockade could be mediated by suppression of acute alterations in the myocardial response to catecholamine stimulation during active ischemia (4). Thus, a short-term increase in beta-adrenergic receptor density (6,37), an augmented adenylate cyclase response to isoproterenol (7) and the potentially toxic effects of increased catecholamine release from sympathetic nerve terminals (38) with possible consequent calcium overload (7) could all be prevented by long-term beta-adrenergic blockade. Other possible mechanisms not examined in the present study include direct antiarrhythmic effects (39), reduction in myocardial oxygen requirements and favorable redistribution of myocardial blood flow during acute ischemia (3,5).

Conclusions. Nevertheless, our study shows that after chronic canine myocardial infarction there is no evidence of increased beta-adrenergic receptor density or augmented (−)-isoproterenol-stimulated adenylate cyclase activity. Whether the potential utility, if any (25,40), of long-term beta-adrenergic blockade after uncomplicated myocardial

infarction is mediated by a pathway other than direct alteration of the beta-adrenergic receptor or coupling between the receptor and adenylate cyclase remains to be demonstrated. If beta-adrenergic blockade is useful after myocardial infarction, its mechanism of action may possibly be related to beneficial alterations in the distribution of myocardial blood flow during ischemia rather than to direct effects on the adrenergic properties of surviving myocardium (41).

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